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INVESTIGATION OF THE ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF DIFFERENT *Carica papaya* PLANT PARTS (SHOOTS, LEAVES, AND SEEDS) EXTRACTED USING DIFFERENT EXTRACTION SOLVENTS

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Abstract
Carica papaya is an important fruit crop in the tropics and is cultivated for consumption in fresh or processed form and for use in the pharmaceutical, leather, and textile industries. Unlike the papaya fruits that are useful for the food industry, the shoots, leaves, and seeds were often discarded as they are considered waste products without realizing their nutrition potential. This study was conducted to determine the *Carica papaya* plant part that yields the highest phenolic compound by the Folin-Ciocalteu method and to find out which *Carica papaya* plant part exhibits the highest antioxidant activity through DPPH free radical scavenging assay. The investigation of antioxidant activity and the total phenolic content of the unused papaya tree parts is significant as the unused parts of the *Carica papaya* tree like shoots, leaves, and seeds can become potential raw materials for the manufacturing of medicine and drugs. The extraction procedure involved two extraction solvents with different ratios which were methanol, ethanol, and a mixture of methanol and ethanol at 1:1, 1:2, and 2:1 ratio respectively. The methanol-extracted shoots of *Carica papaya* yielded the highest phenolic compound with 11.70±0.62 mg GAE/g. While methanol-extracted leaves of *Carica papaya* exhibited the highest antioxidant activity through DPPH free radical scavenging assay with 86.78±0.49%. The present study shows that methanol should be used as the extraction solvent as it is the most efficient solvent in extracting phenolic compounds from the *Carica papaya* plant's part. Extraction using methanol as the solvent could be used for further studies as it can yield maximum phytochemical compounds from the *Carica papaya* plant as well as other plant samples.

Keywords: *Carica papaya*, extraction, antioxidant activity, total phenolic content

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Introduction
Carica papaya belongs to the family Caricaceae and is the most economically important species of the genus *Carica*. It is well known for its edible and nourishing fruit. *C. papaya* is locally known as "betik", but commonly known as papaya, is an easy-to-grow, fast-growing, semi-woody, and usually short-lived plant. Papaya is one of the major tropical crops consumed worldwide either as a vegetable or fresh fruit or as processed products like candy, jam, jellies, and canned. It is an important fruit crop in the tropics and is cultivated for consumption in fresh or processed form and for use in the pharmaceutical, leather, and textile industries (Sanikommu et al., 2021). *C. papaya* fruit has an abundant source of vitamins and minerals in addition to being low in sodium, fats, and calories. The *C. papaya* fruits contain a high level of vitamin A, vitamin C, vitamin E, dietary fibre, and minerals such as calcium, potassium, and sodium

as well as Carotenoids (β-carotene, cryptoxanthin). It was reported that 100 g of fresh and ripe papaya fruits contain approximately 88% water, 10% sugar, 0.5% protein, 0.1% fat, 0.1% acids, 0.6% ash, and 0.7% fibre (Daagema et al., 2020). Unlike the papaya fruits that are useful for the food industry, the shoots, leaves, and seeds were usually discarded as they were considered waste products.

The nutritional value of the unused parts of *C. papaya* like seeds, papaya peel, roots, and leaves are still unfamiliar to the public although there are past studies that propose that the various parts of *C. papaya* have beneficial medicinal properties (Srivastava & Singh, 2016). Chewing the seed of *C. papaya* is said to help in clearing nasal congestion. The green leaves of *C. papaya* have an antiseptic property and the leaf that is processed into tea is reported to help promote digestion. Polyphenols, carotenoids, and traditional antioxidant vitamins such as vitamins C and E may all contribute to the total antioxidant activity of plant materials. Several studies demonstrated that phenolic compounds are the most beneficial bioactive phytochemicals for human health. In fact, some studies have demonstrated a correlation between total phenolic content and antioxidant activity in a variety of seeds, fruits, and vegetables (Addai et al., 2013; Rahayu et al., 2019). Due to its high potential, the investigation of antioxidant activity with 2,2-diphenyl-1-picrylhydrazyl [DPPH] reagent and total phenolic content using the Folin–Ciocalteu method of different *C. papaya* plant parts extract (shoot, leaves, and seeds) must be done in order to maximize the use of papaya resources. The plant parts were extracted using different extraction solvents to determine which solvent is able to extract the most phytochemical compounds. During the past two to three decades, studies have shown that these phytochemicals have a vital role in preventing chronic diseases such as diabetes, cancer, and coronary heart disease (Insanu et al., 2022).

The aim of the study was to measure the total antioxidant activity and the total phenolic content (TPC) from the different parts of papaya which were the seeds, shoots, and leaves using different extraction solvents. DPPH radical scavenging and Folin-Ciocalteu reagents were used to determine the total antioxidant activity and the TPC, respectively of the papaya shoots, leaves and seeds. There were five extraction solvent ratios used which are 100% methanol, 100% ethanol, and a mixture of methanol and ethanol at 1:1, 1:2, and 2:1 ratio (methanol:ethanol) respectively. This study of TPC and antioxidant activity of the papaya seeds, shoots, and leaves is significant as the results of the study can help identify the effect of different extraction solvents and ratios on the TPC and antioxidant activity of *C. papaya*. This can contribute to the increasing database of medicinal plants that could be used as antioxidants in food and medicinal preparations as well as decreasing agricultural waste as more people begin to consume the unused parts of papaya. The outcome of the study might increase the awareness for people to use or consume the unused parts of *C. papaya* like the seeds, shoots, and leaves as they exhibit antioxidant activity and TPC thus lessening the agricultural waste from *C. papaya* plants.

Methods

The preparation of the *Carica papaya* sample (the shoots, leaves, and seeds)

The *C. papaya* seeds, shoots, and leaves were collected from a local house in Kuala Pilah, Negeri Sembilan. The *C. papaya* trees were eight months old when the samples were collected. The *C. papaya* samples were washed thoroughly using tap water several times and rinsed with distilled water to ensure the samples were cleaned. The *C. papaya* shoots and leaves were dried using an oven at 56 °C for 72 hours (Razmavar et al., 2014) while the seeds were dried using an oven at 50 °C for 48 hours (Irondi et al., 2013). The samples were ground using a grinder until they turned into a finely powdered sample. The powdered samples were then sieved to produce a uniform powdered sample, kept in sample bottles and stored in a desiccator.

Carica papaya shoots, leaves, and seeds sample extraction

The plant parts extraction procedure was adapted from a previous study by Asghar et al. (2016). The powdered sample prepared in the previous step was extracted by maceration method using the following five different solvent ratios which were 100% methanol, 100% ethanol, methanol and ethanol with 1:1, 1:2, and 2:1 ratio. The extraction was carried out using a 1:10 (w/v) ratio of *C. papaya* plant part to solvent and the mixture was then left for 2 weeks with periodic shaking at regular intervals. The extraction was then filtered through filter paper before centrifuged at 13000 g for 5 minutes. The filtrates were evaporated with a rotary evaporator until dry. The dried extracts were then reconstituted by adding 5 ml of their respective solvents. The percentage yield of sample extraction was calculated using Equation (1) (Ng et al., 2012).

$$\% \text{ yield} = (\text{weight of dried extract}/\text{weight of dried sample}) \times 100 \quad (1)$$

Determination of DPPH free radical scavenging potential of *Carica papaya* plant parts
DPPH free radical scavenging activity of different extracts of *C. papaya* was determined by following the method described by Asghar et al. (2016). About 1 ml of plant extract with a concentration of 1mg/ml was added to 5 ml methanolic DPPH solution of 0.025g/L concentration. The experiment was carried out in a dark condition as DPPH is light-sensitive. The contents were vortexed for 1 minute and left at room temperature for 20 minutes. The absorbance was measured using a spectrophotometer at 510 nm. The percentage of DPPH inhibition was calculated by using Equation (2).

$$\% \text{ inhibition} = (1-A_1)/A_0 \times 100 \quad (2)$$

Where A_0 is the absorbance of control which is the methanolic DPPH extract with no added extract and A_1 is the absorbance of the extraction solution added with the methanolic DPPH solution scanned at 510 nm.

Determination of total phenolic content of *Carica papaya* plant parts
The procedure for the determination of TPC of the extracts was adapted from a previous study by Radhi Addai et al. (2013). The calibration curve of gallic acid was used for the estimation of the TPC of the samples. 5.0 mg/ml stock standard solution of gallic acid was prepared. The working standards of 2.5, 1.0, 0.5, and 0.1 mg/ml were prepared by diluting the stock solution with distilled water. The absorbance of the solution was measured with a spectrophotometer at 765 nm to plot the standard curve of gallic acid. Next, 4.5 ml distilled water and 0.5 ml diluted Folin-Ciocalteu reagent was added to 100 µl *C. papaya* extracts. The samples were left for 5 minutes before 1 ml of 7.5% Na_2CO_3 was added. The samples were set aside for 2 hours before the absorbances of samples were taken at 765 nm using a spectrophotometer. The results were then compared to the standard curve of gallic acid and represented as mg/gallic acid equivalent (GAE) per g dry matter.

Statistical analysis
Plant extracts from the DPPH free radical scavenging assay and TPC were carried out in triplicates and the data were analyzed using Statistical Product and Service Solutions (SPSS). All the data were expressed as mean±standard deviation. One-way Analysis of Variance (ANOVA) was used to analyze the mean value of the data, and Tukey's post-hoc multiple comparison tests were performed to determine whether there were any statistically significant differences between the means. A probability level of $p<0.05$ was considered statistically significant.

Result and Discussion

Percentage yield of sample extraction
The percentage yield shows the amount of phytochemical compound that was successfully extracted from the dried sample. Table 1 shows the percentage yield of sample extraction that was calculated using Equation (1). The solvent type that is used for extraction is a fundamental factor to consider for optimizing yield extraction. The antioxidants and TPC of plant extracts are significantly influenced by the solvent's polarity, the nature of the extracted compounds, and the extraction technique (Addai et al., 2013). The highest percentage yield can be seen in the leaves of *C. papaya* that were extracted using methanol as a solvent with 35.63% followed by the leaves of *C. papaya* that were extracted using ethanol as a solvent with 33.63% yield.

The results obtained matched the findings by Asghar et al. (2016) which stated that methanol has a higher percentage yield compared to ethanol. The outcome of the study showed that leaves extracted with methanol produced an extraction yield of 15.90 g/100g dry matter followed by ethanol with an extraction yield of 11.47 g/100g dry matter. The seeds of *C. papaya* also exhibited the same pattern as the leaves. The seeds that are extracted with methanol produced an extraction yield of 14.32 g/100g dry matter followed by ethanol with an extraction yield of 12.36 g/100g dry matter. The shoots of *C. papaya* that were extracted using methanol exhibited around 32.14% yield. The poorest yield was achieved with shoots extracted using methanol:ethanol (1:2) with a yield percentage of 12.06%. For the shoots of *C. papaya*, the extraction yield was obtained in the following ascending order; methanol:ethanol (1:2) < methanol:ethanol (1:1) < methanol:ethanol (2:1) < ethanol < methanol. As for the leaves of *C. papaya,* the extraction yield was obtained in the following ascending order; methanol:ethanol (1:2)< methanol:ethanol (1:1)< methanol:ethanol (2:1)<ethanol<methanol. For the seeds of *C. papaya*, the extraction yield was obtained in the following ascending order; methanol:ethanol (2:1)< methanol:ethanol (1:2)< methanol:ethanol (2:1)<ethanol<methanol.

Table 1. Percentage yield of different parts of *C. papaya* in five different extraction solvents.

Plant part of *C. papaya*	Extraction solvent	Weight of dried sample (g)	Weight of dried extract (g)	Percentage yield (%)
	Methanol	5	1.6070	32.14
	Ethanol	5	1.1090	22.18
Shoots	Methanol:Ethanol (1:1)	5	0.6855	13.71
	Methanol:Ethanol (2:1)	5	0.8400	16.80
	Methanol:Ethanol (1:2)	5	0.6030	12.06
	Methanol	5	1.7815	35.63
	Ethanol	5	1.6815	33.63
Leaves	Methanol:Ethanol (1:1)	5	1.0595	21.19
	Methanol:Ethanol (2:1)	5	1.4345	28.69
	Methanol:Ethanol (1:2)	5	0.6355	12.71
	Methanol	5	1.5870	31.74
	Ethanol	5	1.4480	28.96
Seeds	Methanol:Ethanol (1:1)	5	1.3165	26.33
	Methanol:Ethanol (2:1)	5	0.9595	19.19
	Methanol:Ethanol (1:2)	5	1.0495	20.99

The extraction method, temperature, extraction duration, phytochemical composition, and solvent all have a significant impact on the extraction's efficiency. A solvent is considered one of the most crucial extraction process parameters (Truong et al., 2019). Different solvents resulted in varied extraction yields, as demonstrated by the findings. This is because differences in the solvent polarity of the extraction solvents could result in the extraction of a wide variety of bioactive component concentrations.

C. papaya plants extracted with methanol had a higher extraction yield than those extracted with ethanol, indicating that the extraction efficiency prefers highly polar solvents. This could be due to the plant material's high concentration of polar molecules that are more soluble in methanol than ethanol. Methanol's boiling point is 64.7 °C, which is lower than ethanol's boiling point (78.37 °C), which makes it easy to evaporate after the extraction process thus increasing the percentage yield of sample extraction. The polarity of solvents also affected the result of the percentage yield of the sample extraction. The higher the polarity, the higher the extraction yield (Nawaz et al., 2020). The polarity of solvents was determined using the dielectric constant (δ), where the dielectric constant of a compound is described as its polarity index. Greater δ will result in a higher polarity (Abd Aziz et al., 2021). Methanol has a dielectric constant of 32.7 which is higher than ethanol with a 24.5 dielectric constant. The polarity of solvents is very important to increase the solubility of antioxidant compounds and the TPC. These findings reveal that methanol is the optimal solvent for extracting bioactive components from the shoots, leaves, and seeds of *C. papaya*. Extraction solvents influence extraction yield and bioactive component content, hence considerably altering the biological activity of the extract (Truong et al., 2019)

Determination of DPPH free radical scavenging potential of *Carica papaya* parts sample extracted using different solvents

The determination of DPPH free radical scavenging potential was calculated using Equation (2). The DPPH assay is a simple and quick method to measure the antioxidant's capacity to scavenge free radicals since it uses a stable free radical. It provides reliable data on the antioxidant capacity of the tested compounds to act as hydrogen donors or free radical scavengers (Halliwell, 2011; Ye et al., 2015). Table 2 shows the percentage inhibition of DPPH free radical scavenging potential of the shoots, leaves, and seeds of *C. papaya* extracted with five different solvent ratios which are methanol, ethanol, and a mixture of different ratios of methanol and ethanol which are methanol:ethanol (1:1), methanol:ethanol (2:1) and methanol:ethanol (1:2).

The DPPH was significantly different between different types of solvent for one sample. The leaves of *C. papaya* extracted with methanol exhibit the highest percentage inhibition of DPPH free radical scavenging potential (86.78±0.49%) while the seeds of *C. papaya* extracted with methanol:ethanol (1:2) exhibit the lowest percentage inhibition of DPPH free radical scavenging potential (27.70±2.34%). This finding corresponds with past studies by Maisarah et al. (2013) where the leaves of *C. papaya* have a higher scavenging effect compared to other parts of the plant with the EC_{50} value of 7.8±0.01mg/ml. The study also agrees with Maisarah et al. (2013) that the seeds of *C. papaya* exhibit the lowest scavenging effect compared to other parts of the plant with the EC_{50} value of 1.0±0.01mg/ml. A previous study by Nisa et al. (2019) also recorded that the leaves of *C. papaya* have a higher antioxidant activity when extracted using methanol compared to ethanol, the percentage inhibition of DPPH free radical scavenging potential from leaves extracted using methanol was recorded as 77.40% while ethanol has a 69.71% of percentage inhibition of DPPH free radical scavenging potential. The shoots and the leaves of *C. papaya* that are extracted using methanol both show a higher percentage inhibition of DPPH free radical scavenging potential which are 66.98% and 56.42% compared to other extraction solvents.

The ANOVA analysis of the solvent used in extracting the shoots, leaves, and seeds of *C. papaya* was statistically significant ($p<0.01$) indicating that the percentage inhibition of DPPH free radical scavenging potential of the plant parts was influenced by the solvent used during the extraction process. The results correspond with the study by Addai et al. (2013) where it was concluded that the best solvent for producing extracts with high antioxidant activities in both papaya fruit cultivars was 50% methanol. However, the ethanolic extracts of shoots and the leaves have indicated the lowest percentage of inhibition which were 52.78% and 77.86% compared to the seeds where the solvent methanol:ethanol (1:2) exhibit the lowest percentage.

Table 2. Percentage inhibition of DPPH free radical scavenging potential of the different parts of *C. papaya* extracted with five different solvent ratios (Mean±SD; n=3)

Plant part of *C. papaya*	Extraction solvent	% Inhibition of DPPH free radical scavenging potential
Shoots	Methanol	66.98±1.45
	Ethanol	52.78±0.36
	Methanol:Ethanol (1:1)	57.94±1.26
	Methanol:Ethanol (2:1)	63.58±2.91
	Methanol:Ethanol (1:2)	56.46±2.12
Leaves	Methanol	86.78±0.49
	Ethanol	77.86±0.91
	Methanol:Ethanol (1:1)	85.33±0.12
	Methanol:Ethanol (2:1)	86.23±0.38
	Methanol:Ethanol (1:2)	79.03±1.87
Seeds	Methanol	56.42±1.03
	Ethanol	33.14±2.60
	Methanol:Ethanol (1:1)	33.57±2.95

Methanol:Ethanol (2:1)	42.61±8.89
Methanol:Ethanol (1:2)	27.70±2.34

Values are expressed as mean ±standard deviation (n=3)

Figure 1 shows the means of the percentage inhibition of DPPH free radical scavenging potential of the *C. papaya* plant part extracts extracted using different solvents. For the shoots of *C. papaya*, the percentage inhibition of DPPH free radical scavenging potential was obtained in the following descending order; methanol> methanol:ethanol (2:1)> methanol:ethanol (1:1)> methanol:ethanol (1:2)> ethanol. A similar pattern can also be recognized in the leaves of *C. papaya* where the percentage inhibition of DPPH free radical scavenging potential was obtained in the following descending order; methanol> methanol:ethanol (2:1)> methanol:ethanol (1:1)> methanol:ethanol (1:2)> ethanol. The percentage inhibition of DPPH free radical scavenging potential from the seeds of *C. papaya* was obtained in the following descending order; methanol> methanol:ethanol (2:1)> methanol:ethanol (1:1)> ethanol>methanol:ethanol (1:2)

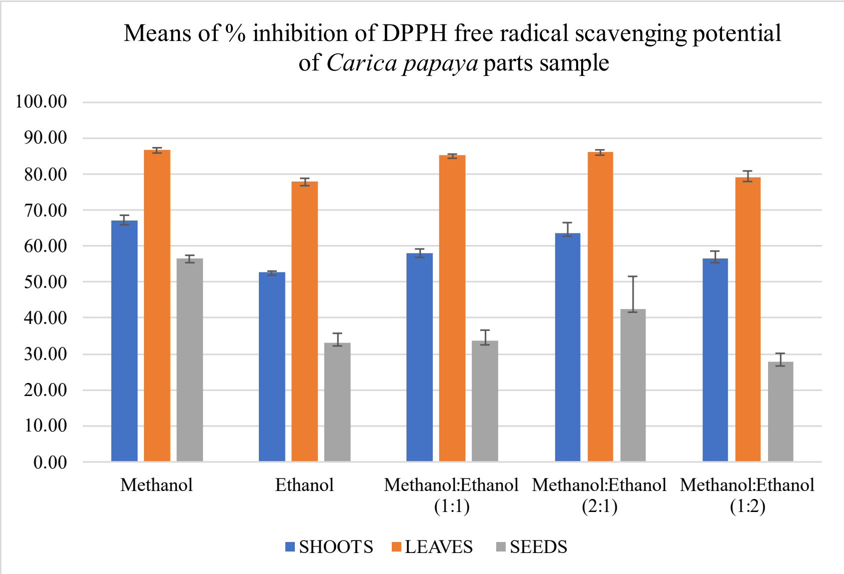

Figure 1. The means of % inhibition of DPPH free radical scavenging potential of *C. papaya* parts sample

Total phenolic contents (TPC) of *Carica papaya* parts sample extracted using different solvents

Phenolic compounds are abundantly present in plants and have drawn a lot of attention because of their antioxidant and free radical-scavenging properties, which may have beneficial effects on human health. The TPC was determined by a standard method using Folin-Ciocalteu reagent in comparison with standard gallic acid and the results were expressed in terms of mg gallic aid equivalent (GAE)/ g dry sample.

The one-way between-group analysis of variance (ANOVA) was used to investigate the impact of extraction solvent used towards the TPC of the shoots and seeds of *C. papaya*. The ANOVA of the solvent used in extracting the shoots and seeds of *C. papaya* was statistically significant at ($p<0.01$) indicating that the TPC of the plant parts were influenced by the solvent used during the extraction process. However, the ANOVA analysis of the solvent used in extracting the leaves of *C. papaya* was not statistically significant at ($p>0.01$). This could be possibly due to an error that occurred during the extracting period or when preparing the sample for the determination of the TPC. A linear calibration curve of gallic acid with an R^2 value of 0.9468 was obtained as shown in Figure 2.

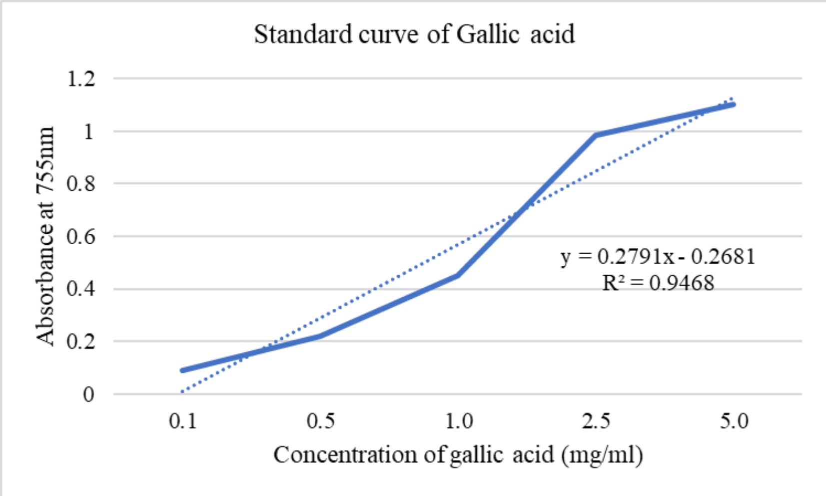

Figure 2. The standard curve of Gallic acid

It can be observed in Table 3 that the shoots of *C. papaya* extracted with methanol exhibited the highest TPC (11.69±0.62 mg GAE/g) while the seeds of *C. papaya* extracted with ethanol exhibited the lowest TPC (3.12±0.02 mg GAE/g). This finding corresponds with the past studies by Maisarah et al. (2013) which recorded that *C. papaya* shoots (333.14 ±11.02 mg GAE/100g) have a higher TPC compared to the seeds (59.54 ±12.23 mg GAE/100g). The leaves and the seeds of *C. papaya* that were extracted using methanol both show a higher TPC which were 10.41±0.61 and 6.94±0.27 mg GAE/g compared to the same plant parts that were extracted using other extraction solvents. The results were in agreement with the study by Getal et al. (2020) where the concentration of TPC present in the leaf of *C. papaya* extracted using methanol is higher than in the leaf of *C. papaya* extracted using ethanol with 13.7 mg GAE/g and 13.5 mgGAE/g. However, the shoots and the seeds of *C. papaya* that are extracted using ethanol indicate the lowest TPC which are 8.08±0.12 and 3.12±0.02 mg GAE/g compared to the leaves where the solvent methanol:ethanol (1:2) exhibit the lowest percentage. For the shoots of *C. papaya*, the TPC was obtained in the following descending order; methanol> methanol:ethanol (1:1)> methanol:ethanol (2:1)> methanol:ethanol (1:2)> ethanol. A similar pattern can also be recognised in the seeds of *C. papaya* where the TPC was obtained in the following descending order; methanol> methanol:ethanol (1:1)> methanol:ethanol (2:1)> methanol:ethanol (1:2)> ethanol. The TPC from the leaves of *C. papaya* was obtained in the following descending order; methanol> methanol:ethanol (2:1)> methanol:ethanol (1:1)> ethanol>methanol:ethanol (1:2).

Table 3. The TPC of *C. papaya* parts sample extracted with five different solvent ratios (Mean±SD; n=3)

Plant part of *C. papaya*	**Extraction solvent**	**TPC mg GAE/g**
Shoots	Methanol	11.69±0.62
	Ethanol	8.08±0.12
	Methanol:Ethanol (1:1)	10.46±0.31
	Methanol:Ethanol (2:1)	10.56±0.10
	Methanol:Ethanol (1:2)	9.58±0.17
Leaves	Methanol	10.41±0.61
	Ethanol	9.62±0.28
	Methanol:Ethanol (1:1)	9.68±0.35
	Methanol:Ethanol (2:1)	9.72±0.31
	Methanol:Ethanol (1:2)	9.59±0.33
Seeds	Methanol	6.94±0.27
	Ethanol	3.12±0.02
	Methanol:Ethanol (1:1)	6.82±0.09
	Methanol:Ethanol (2:1)	5.07±0.15
	Methanol:Ethanol (1:2)	3.73±0.10

Values are expressed as mean ±standard deviation (n=3)

Figure 3 shows the mean of TPC of the *C. papaya* parts sample using the GAE equation of y = 0.2791x - 0.2681 (R^2 =0.9468), whereby y = absorbance of the sample at 765nm and x = concentration of TPC in mg GAE/g of the extract.

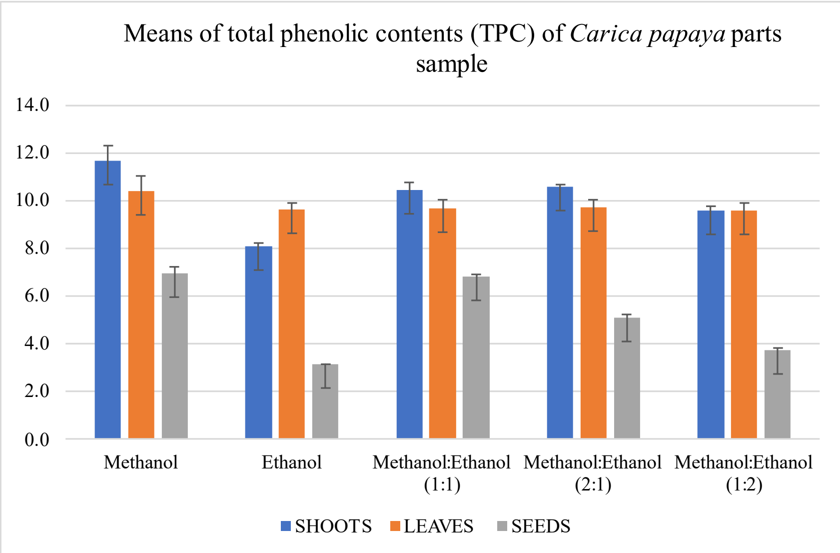

Figure 3. The means of TPC of *C. papaya* parts sample

Conclusion

In conclusion, the methanolic extracts of *C. papaya* shoots yielded the highest phenolic compound of 11.70±0.62 mg GAE/g while the methanolic extract of *C. papaya* leaves exhibited the highest antioxidant activity through DPPH free radical scavenging assay with 86.78±0.49%. Methanol was found to be the best solvent of choice to extract the phytochemical compound from *C. papaya* plant parts. The findings of this study further prove that the unconsumed plant parts of *C. papaya* indeed contain beneficial nutrients and can be a source for the development of medicines.

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Author Contribution

NS Mohd Rozy – Conceptualization, data curation and writing draft; MA Ibrahim – Supervision, writing- review and editing.

Conflict of Interest

The author declares no conflict of interest.

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